amplification reaction to a test solution, wherein an amplified product is labeled with a marker molecule. The method comprises:

(a) performing a nucleic acid amplification reaction of the target nucleic acid in a test solution containing a forward primer and a reverse primer, a substrate comprising nucleotides, a nucleic acid polymerase and a target nucleic acid molecule, wherein the number of one of the forward primer and the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal;

(b) measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

(c) evaluating a fluctuation motion of the amplified nucleic acid which is labeled with the marker molecule, in the test solution on the basis of the signal detected; and

(d) quantifying the target nucleic acid molecule on the basis of evaluation results.

Important features of the present invention reside in that the number of the forward primer or the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal. According to applicant's method, an amplified double stranded nucleic acid product is labeled with a marker

molecule without fail, whereas a single-stranded nucleic acid product is not labeled (see Fig. 3 of the present application). The reaction is observed by fluorescence correlation spectroscopy ("FCS"). In this manner, the present invention makes it possible to analyze a target nucleic acid.

Claims 1 to 5, 7, 8, 39 and 42 to 43 were rejected under 35 USC 103 as being obvious over Eigen et al. USP 5,807,677 in view of Gyllensten et al., Proc. Natl. Acad. Sci. USA, 85, 7625-7656, (1988) and Wang et al. USP 5,567,583 for the reasons set forth in Paragraph No. 6 on pages 3 and 4 of the Office Action.

In the Office Action, it was asserted that the method of Eigen et al. involves FCS in which the target nucleic acid is amplified with PCR. However, this contention is not correct. Eigen et al., in column 3, lines 46 to 57, discuss only the advantages of FCS over the polymerase chain reaction ("PCR").

Further, the Office Action alleged that the method of Eigen et al. inherently uses a forward primer and a reverse primer. However, this statement is also not correct. As is clear from Fig. 1 in Eigen et al., primers 12, 13 and 14 are all directed in the same direction, and thus two types of primers in opposite directions are not used. Basically, as discussed in column 4, lines 4 to 5 of Eigen et al., this reference uses FCS in order to overcome the defect of PCR that uses two types of primers, namely a forward primer and a reverse primer. It should be noted that

the term "primer" used in Eigen et al. does not indicate a primer used in PCR. The primer discussed in Eigen et al., column 2, lines 30 to 38, referred to in the Office Action, is merely a labeled nucleic acid probe used only for labeling a target nucleic acid by hybridizing with it. The Eigen et al. method does not involve a PCR amplification reaction using forward and reverse primers.

The Office Action admitted that Gyllensten et al. do not disclose that the primer which has a low concentration is labeled.

Gyllensten et al. disclose an asymmetric PCR. However, as is clear from, for example, the Abstract of Gyllensten et al., the object of Gyllensten et al. is to produce a great amount of single-stranded DNA and a small amount of double-stranded DNA by PCR amplification (see page 25, lines 10 to 18 of the present specification). Thus, the general object of the asymmetric PCR in Gyllensten et al. is to obtain single-stranded DNA to be used as a probe or as a subject of sequencing. For this object, it suffices only if a single strand is separated from a double strand by electrophoreses after carrying out an asymmetric PCR, and it is entirely unnecessary to label a primer with a fluorescent material. Therefore, the combination of Gyllensten et al. and FCS is not taught or suggested.

It was contended in the Office Action that a person with ordinary skill in the art would have also labeled the primer which has a low concentration in the reaction. However, there is no basis for this allegation for the following reason. As discussed above, it is not necessary in Gyllensten et al. to label a primer with a fluorescent material, and therefore it is only natural that Gyllensten et al. do not teach or suggest labeling a primer that has a low concentration.

The labeling of a primer having a low concentration in the asymmetric PCR method is advantageous only in the case where double-stranded DNA created by the asymmetrical PCR is to be detected, especially in the case where double-stranded DNA that is initially present in the sample is subjected to quantitative analysis by detecting it with, especially, the FCS method. In this case, the quantity of the target DNA can be determined from the correlation between the amount of the labeled primer that has a low concentration, the amount of the target double-stranded nucleic acid present in the sample and the amount of labeled double-stranded DNA produced by the PCR reaction (see page 28, line 27 to page 29, line 5 of the present specification).

By contrast, Gyllensten et al. has the object of obtaining a single-stranded DNA and, therefore, it is not at all necessary to label only double-stranded products. Therefore, one of ordinary skill in the art would not consider that Gyllensten et al.

suggest the labeling of a minor primer with a fluorescent material.

Wang et al. do not disclose labeling of a primer with a fluorescent material.

It is therefore respectfully admitted that applicant's claimed invention is not rendered obvious over the references, either singly or combined in the manner relied upon in the Office Action in view of the distinctions discussed hereinabove. It is furthermore submitted that there are no teachings in the references to combine them in the manner relied upon in the Office Action.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,

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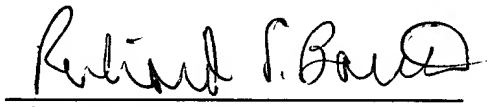

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